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Evaluation of MAP-specific peptides following vaccination of goats

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Our aim is to develop a subunit MAP vaccine not interfering with the diagnosis of paratuberculosis or bovine tuberculosis. This study's objective was to evaluate MAP-specific peptides defined by *in silico* analysis.

Peptides were picked by 1) comparing MAP genomes to that of other mycobacterium species or 2) selected based on "experience". Peptides predicted to bind bovine MHC II by *in silico* analysis were included in further studies, resulting in two panels 1) genome-based and 2) selected. Initially, two groups of 15 healthy goats were vaccinated with one of the two panels (50 µg/peptide in CAF01 adjuvant/CAF04 for boosting). Four MAP-infected goats were also vaccinated. In a second vaccination trail, groups of 8 healthy goat kids were vaccinated with genome-based peptides, selected peptides or selected peptides linked together in a recombinant protein (20 µg/peptide or 50 µg protein in Montanide ISA61 adjuvant). IFN-γ responses were measured by ELISA and ELISPOT upon stimulation with peptide pools or individual peptides. T cell lines were made by cultivating CD4+ cells in the presence of antigen, feeder cells plus cytokines, and used to evaluate responses to peptide pools and individual peptides.

IFN-γ responses in healthy goats after the first vaccination were low, but testing of T cell lines from MAP-infected goats identified peptides inducing strong proliferative responses. Peptides for a second vaccination were selected by combining results from this study with a parallel cattle study. In the second trial, goats in the genome-based and the selected peptide group had solid IFN-γ responses while goats in the protein group had modest responses. Only a moderate boosting effect was seen in the second trail. The genome-based pool induced the strongest CD4+ T cell line responses and had the highest number of immunogenic peptides.

This study shows 1) that detection of immunogenic antigens using *in silico* predictions and T cell lines work, and 2) the identified MAP-specific peptides show potential for use in a subunit vaccine.